

5 **WHAT IS CLAIMED IS:**

1. A composition comprising at least two nanoparticle conjugates,
each nanoparticle conjugate comprising:
 a magnetic nanoparticle; and
 at least one substrate moiety, in which each substrate moiety is linked to
10 the nanoparticle and is chemically modified when the conjugate interacts with a
target enzyme; wherein,
 when the target enzyme is absent, the nanoparticle conjugates are
essentially monodisperse in a liquid; and
 when the target enzyme is present, the nanoparticle conjugates self-
15 assemble into one or more nanoparticle conjugate clusters through the formation
of intermolecular linkages between the chemically modified substrate moieties.
2. The composition of claim 1, wherein the the conjugates further
comprise functional groups that link the nanoparticle to one or more substrate
20 moieties.
3. The composition of claim 2, wherein the functional groups are
selected from amino, $\text{-NHC(O)(CH}_2\text{)}_n\text{C(O)-}$, carboxy, or sulfhydryl groups,
wherein n is 0-100.
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4. The composition of claim 1, wherein the magnetic nanoparticles
each comprise a magnetic metal oxide.
5. The composition of claim 4, wherein the magnetic metal oxide is
30 a superparamagnetic metal oxide.
6. The composition of claim 4, wherein the metal oxide is iron
oxide.
- 35 7. The composition of claim 4, wherein the nanoparticles are an
amino-derivatized cross-linked iron oxide nanoparticles.

- 5 8. The composition of claim 1, wherein the substrate moieties
comprise a phenolic moiety.
9. The composition of claim 1, wherein the substrate moieties are
chemically modified by oxidation.
- 10 10. The composition of claim 9, wherein the oxidation is a one
electron oxidation.
11. The composition of claim 1, wherein the target enzyme is a
15 protease.
12. The composition of claim 1, wherein the target enzyme is a
peroxidase.
- 20 13. The composition of claim 12, wherein the peroxidase is
myeloperoxidase.
14. The composition of claim 12, wherein the peroxidase is
horseradish peroxidase.
- 25 15. The composition of claim 1, wherein each of the monodisperse
nanoparticle conjugates has an average particle size of between about 40 nm and
about 60 nm.
- 30 16. The composition of claim 1, wherein each of the monodisperse
nanoparticle conjugates has an average particle size of about 50 nm.
17. The composition of claim 1, wherein each of the nanoparticle
conjugate clusters has an average particle size of between about 400 nm and
35 about 500 nm.
18. The composition of claim 1, wherein each of the nanoparticle
conjugate clusters has an average particle size of about 450 nm.

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19. The composition of claim 14, wherein each of the monodisperse nanoparticle conjugates has an R1 relaxivity between about 5 and 30 mM⁻¹ sec⁻¹ and an R2 relaxivity between about 15 and 100 mM⁻¹ sec⁻¹.

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20. The composition of claim 1, wherein the intermolecular linkages are covalent linkages.

21. The composition of claim 1, wherein the intermolecular linkages are non-covalent linkages.

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22. The composition of claim 1, wherein the formation of intermolecular linkages between the chemically modified substrate moieties is irreversible.

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23. The composition of claim 1, wherein the formation of intermolecular linkages between the chemically modified substrate moieties results in crosslinking of the nanoparticle conjugates.

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24. The composition of claim 1, wherein the composition further comprises a fluid media.

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25. The composition of claim 24, wherein self-assembly of the nanoparticle conjugates results in the spin-spin relaxation time of the fluid being decreased relative to the spin-spin relaxation time of the fluid having essentially only monodisperse nanoparticle conjugates present.

26. The composition of claim 24, wherein the decrease in spin-spin relaxation time is dependent upon the concentration of the target enzyme.

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27. The composition of claim 1, wherein the nanoparticle conjugate has a formula

X-(L)_x-A, wherein:

X is a magnetic nanoparticle;

5 L is -NH-, -NHC(O)(CH₂)_nC(O)-, -C(O)O-, or -SS-, wherein n is 0-20;

A is substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, substituted or unsubstituted heteroaralkyl, substituted or unsubstituted aralkylamino, or substituted or
10 unsubstituted heteroaralkylamino; wherein substituents are selected from halo, hydroxy, C₁-C₄ alkoxy, or C₁-C₄ alkyl; and
x is 0 or 1.

28. The composition of claim 27, wherein X is magnetic metal oxide.
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29. The composition of claim 28, wherein the metal oxide is iron oxide.

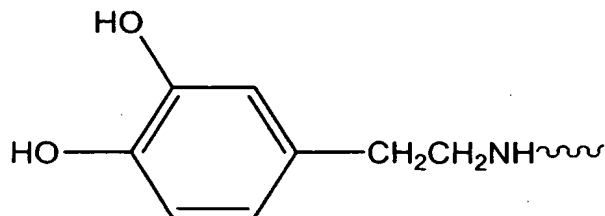
30. The composition of claim 27, wherein x is 1 and L is -
20 NHC(O)(CH₂)_nC(O)-.

31. The composition of claim 30, wherein n is 6.

32. The composition of claim 27, wherein A is substituted
25 aralkylamino, or substituted heteroaralkylamino.

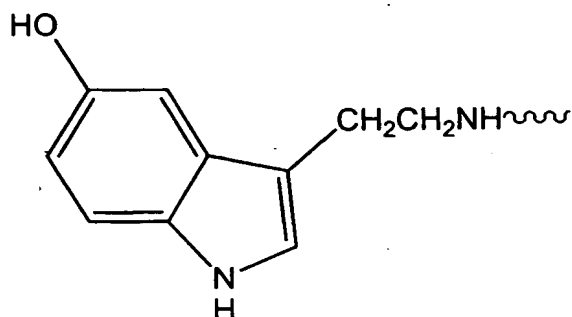
33. The composition of claim 32, wherein A is substituted with at least one hydroxyl group.

30 34. The composition of claim 33, wherein A is:



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35. The composition of claim 33, wherein A is:



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36. An *in vitro* method for detecting the presence of a target enzyme in a sample, the method comprising:

(i) providing a composition comprising at least two nanoparticle conjugates, each nanoparticle conjugate comprising a magnetic nanoparticle; and at least one substrate moiety, in which each substrate moiety is linked to the nanoparticle and is chemically modified when the conjugate interacts with a target enzyme; wherein, when the target enzyme is absent, the nanoparticle conjugates are essentially monodisperse; and when the target enzyme is present, the nanoparticle conjugates self-assemble into one or more nanoparticle conjugate clusters through the formation of intermolecular linkages between the chemically modified substrate moieties;

(ii) contacting the composition with a fluid sample;

(iii) allowing time (a) for the target enzyme to contact the nanoparticle conjugates and (b) for the nanoparticle conjugates to self-assemble into clusters through the formation of intermolecular linkages between the chemically modified substrate moieties; and

(iv) determining the spin-spin relaxation time of the fluid over time,

wherein a decrease in spin-spin relaxation time indicates the presence of the target enzyme in the sample.

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37. The method of claim 37, further comprising the addition of hydrogen peroxide.

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38. The method of claim 36, further comprising the addition of glucose oxidase.

39. An *in vivo* method for detecting the presence of a target enzyme in a subject, the method comprising:

10 (i) administering to the subject a composition comprising at least two nanoparticle conjugates, each nanoparticle conjugate comprising a magnetic nanoparticle; and at least one substrate moiety, in which each substrate moiety is linked to the nanoparticle and is chemically modified when the conjugate

15 interacts with a target enzyme; wherein, when the target enzyme is absent, the nanoparticle conjugates are essentially monodisperse; and when the target enzyme is present, the nanoparticle conjugates self-assemble into one or more nanoparticle conjugate clusters through the formation of intermolecular linkages between the chemically modified substrate moieties;

20 (ii) allowing time (a) for the target enzyme to contact the nanoparticle conjugates and (b) for the nanoparticle conjugates to self-assemble into clusters through the formation of intermolecular linkages between the chemically modified substrate moieties; and

(iii) determining the spin-spin relaxation time of the fluid over

25 time,

wherein a decrease in spin-spin relaxation time indicates the presence of the target enzyme in the subject.

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40. The method of claim 39, wherein the subject is a human.

41. The method of claim 39, further comprising the step of identifying the subject as being in need of such detection.

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42. A self-assembling, nanoparticle conjugate comprising:
a magnetic nanoparticle; and
at least one substrate moiety, in which each substrate moiety is linked to
the nanoparticle and is chemically modified when the conjugate interacts with a
target enzyme; wherein,
when two or more nanoparticle conjugates are present and when the
target enzyme is absent, the nanoparticle conjugates are essentially
monodisperse in a liquid; and
when two or more nanoparticle conjugates are present and when the
target enzyme is present, the nanoparticle conjugates self-assemble into one or
more nanoparticle conjugate clusters through the formation of intermolecular
linkages between the chemically modified substrate moieties.

43. The nanoparticle conjugate of claim 42, wherein the conjugate
has a formula $X-(L)_x-A$,
wherein:
X is a magnetic nanoparticle;
L is $-NH-$, $-NHC(O)(CH_2)_nC(O)-$, $-C(O)O-$, or $-SS-$, wherein n is
0-20;
A is substituted or unsubstituted aryl, substituted or unsubstituted
heteroaryl, substituted or unsubstituted aralkyl, substituted or unsubstituted
heteroaralkyl, substituted or unsubstituted aralkylamino, or substituted or
unsubstituted heteroaralkylamino; wherein substituents are selected from halo,
hydroxy, C_1-C_4 alkoxy, or C_1-C_4 alkyl; and
x is 0 or 1.

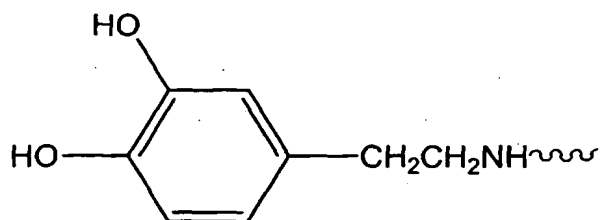
44. The conjugate of claim 43, wherein X is magnetic metal oxide.
45. The conjugate of claim 44, wherein the metal oxide is iron oxide.
46. The conjugate of claim 43, wherein x is 1 and L is $-NHC(O)(CH_2)_nC(O)-$.

5 47. The conjugate of claim 46, wherein n is 6.

 48. The conjugate of claim 43, wherein A is substituted aralkylamino,
or substituted heteroaralkylamino.

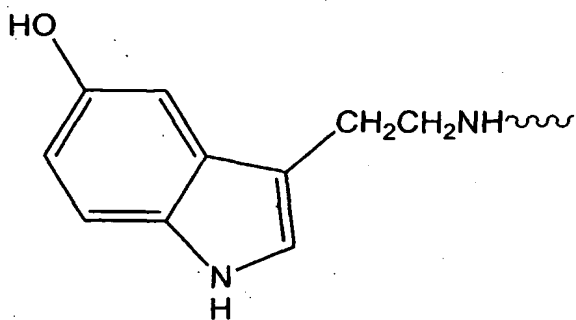
10 49. The conjugate of claim 48, wherein A is substituted with at least
one hydroxyl group.

 50. The composition of claim 49, wherein A is:



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 51. The conjugate of claim 49, wherein A is:



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- 5 52. A packaged product comprising:
 a composition comprising at least two nanoparticle conjugates, each
nanoparticle conjugate comprising:
 a magnetic nanoparticle; and
 at least one substrate moiety, in which each substrate moiety is linked to
10 the nanoparticle and is chemically modified when the conjugate interacts with a
target enzyme; wherein,
 when the target enzyme is absent, the nanoparticle conjugates are
essentially monodisperse in a liquid; and
 when the target enzyme is present, the nanoparticle conjugates self-
15 assemble into one or more nanoparticle conjugate clusters through the formation
of intermolecular linkages between the chemically modified substrate moieties.